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HYDROGEN-ION CONCENTRATION IN ITS RELATION TO WHEAT SCAB [1]

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That the reaction of the soil affects the severity of certain soil-borne plant diseases such as potato scab and the club-root of cabbage has been known for many years. That the hydrogen-ion concentration of the soil solution, however, is the controlling factor was not appreciated until Gillespie (1, 2) demonstrated that the soil solution has a definite hydrogen-ion concentration which can be easily determined, and Gillespie and Hurst (3, 4) showed that the hydrogen-ion concentration of the soil solution is apparently the controlling factor in the incidence of potato scab. The demonstration of the close relation between hydrogen-ion concentration and this soil-borne disease suggests that a complete and thorough investigation should be made of the relation of hydrogen-ion concentration to the development of other pathogenic organisms carried in the soil and to their ability to infect the host. In the present paper, a study is presented of the relation of hydrogen-ion concentration to the growth of *Gibberella Saubinetii*, the causal organism of wheat scab, and to the ability of this organism to produce seedling infection in wheat.

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF GIBBERELLA SAUBINETII (MONT.) SACC.

Comparatively little work has been done on the relation of hydrogen-ion concentration to the growth of pathogenic or non-pathogenic fungi. Clark and Lubs (5) grew *Aspergillus niger* on a mineral nutrient solution plus sucrose and found that the hydrogen-ion concentration on the seventh day was 2×10^{-2} ($p_H = 1.70$). Duggar, Severy, and Schmitz (6), in studying certain fungi in plant decoctions, found that in all solutions they used except sugar-beet and cornmeal decoctions, *Aspergillus niger* caused a shift towards the acid side equivalent to a hydrogen-ion concentration of about 10^{-3} ($p_H = 3.0$), while *Macrosporium commune* and *Glomerella Gossypii* generally evidenced a pronounced change in the other direction. With these latter two organisms the reactions of turnip, beet, and potato decoctions were changed from 10^{-6} to 10^{-8} ($p_H = 6.0$ to 8.0). The authors state that

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acidity was developed by Penicillium when sugar was added to the decoction, but that alkalinity was developed by the same organism when no sugar was added. Working with four wood-rotting fungi, Meacham (7) found that these organisms grew over a large range of acidity and that a rather high concentration of the hydrogen ion is required to inhibit their growth. He places the limiting acidity at a p_H of about 1.7. His graph shows that in proceeding towards a more acid reaction, growth is fairly uniform until a p_H of about 2.6 is reached, when growth begins to decrease very rapidly to p_H 1.9. It then decreases more gradually to p_H 1.7. He states that frequently there is a maximum of growth which occurs at about p_H 3.

Brightman, Meacham, and Acree (8) in their investigations of "salt effects" and phenolsulphonphthalein indicators illustrate graphically some data obtained by Meacham on the growth of *Endothia parasitica* at various hydrogen-ion concentrations on bean decoction buffered with dipotassium hydrogen phosphate and acetic acid. As drawn, their curve for the initial p_H of the medium plotted against growth increases to a maximum at about p_H 4.5, falls off to about p_H 5.0, and then continues practically horizontally to p_H 8.0 where it descends rapidly to p_H 8.5. If this curve were redrawn through the points as plotted following the points more closely between p_H 4.0 and p_H 6, it would be seen that the maximum at p_H 4.5 is followed by a distinct minimum and a second maximum. Similarly, the curve of the final p_H of the medium plotted against the growth shows slight evidence of a minimum. Attention is drawn to this minimum, apparently overlooked by these workers, because of certain data presented in this paper which show a minimum in the growth of *Gibberella Saubinetii* as plotted against hydrogen-ion concentration.

Meacham, Hopfield, and Acree (9) grew *Endothia parasitica* on various media in which the hydrogen-ion concentration was regulated by means of phosphate-acetate and phosphate-phthalate buffer mixtures. They found that the organism grew well at a p_H of about 5.7.

The above named organisms all apparently have a rather wide range of acidity at which they will grow. In the case of the potato-scab organisms, however, Gillespie (10) found that the growth is inhibited by a relatively low acidity. All the strains he used showed inhibition at p_H 5.1, and usually there was no growth at all at p_H 4.8. Waksman (11) found that the limiting concentrations for the genus Actinomyces as a whole are about p_H 5.0 and p_H 9.0, although several species are able to grow at higher hydrogen-ion concentrations (p_H 4.6–4.8). The optimum reaction is placed at p_H 7.0–7.8.

Zeller, Schmitz, and Duggar (12) conclude from their work on wood-destroying fungi on liquid media that the hydrogen-ion concentration is not a limiting factor in the growth of these organisms. Webb (13) presents some interesting data on the relation of hydrogen-ion concentration to the germination of the spores of certain fungi. He determined the maximum

and limiting acidities for the germination of the spores of *Aspergillus niger*, *Penicillium cyclopium*, *Botrytis cinerea*, and a species of Fusarium. His results with *Fusarium* sp. seem especially significant in connection with the data presented in this paper. Webb found with the spores of this fungus that at 22° after 20 hours no germination occurred at p_H 2.8, slight germination at 3.1, and increasing germination up to 5.0. From this point the germination falls off to 6.2, rises to a second high point at 7.4, and finally declines to 10.0. The curve therefore has two maxima with a minimum between. Webb's curve for the germination of the spores of *Fusarium* sp. at 27° also shows a distinct minimum at p_H 6.2. He also obtained minimum points, though less marked, in the germination curves of the spores of *Aspergillus niger* at 27° C. and of *Penicillium cyclopium* at 27° and 31° C.

In the present study, the effect of hydrogen-ion concentration on the growth of *Gibberella Saubinetii*, the causal organism of wheat scab, was determined. An authentic culture of the pathogene was secured from Professor J. G. Dickson of the University of Wisconsin. A single spore isolation from this culture was used throughout the studies here presented. Three experiments were performed. In the first the reaction of a liquid medium was adjusted with sulphuric acid and sodium hydroxide, in the second with phosphates, phosphoric acid, and potassium hydroxide, and in the third lactic acid was used with potato-dextrose agar.

Experiment 1. Growth on Liquid Media—Sulphuric-Acid-Sodium-Hydroxide Series

The culture solution used had the following composition:

KNO_3	2.0 g.
$MgSO_4.7H_2O$	0.5 g.
KH_2PO_4	0.1 g.
Glucose	10.0 g.
H_2O	1,000 cc.

Fifty cubic centimeters of this solution were added to each culture flask. Erlenmeyer Pyrex flasks of 150-cubic-centimeter capacity were used. The flasks containing the solution were sterilized, and the reaction was adjusted when they were cool by adding a varying number of drops of sterile solutions of $N/1$ and $N/10$ H_2SO_4 and $N/1$ and $N/10$ NaOH. Five cubic centimeters of the solution were then withdrawn aseptically from each flask for the determination of the hydrogen-ion concentration. The colorimetric method devised by Gillespie (2) was used in obtaining the p_H values. The cultures were inoculated with mycelium cut from potato-agar plates. They were incubated in a dark room at a temperature of 26° C. At the end of seven days, ten cubic centimeters of concentrated hydrochloric acid were added to each flask to stop the growth. The dry weights of the mycelial mats were determined by filtering into a Gooch crucible and drying at 110° C. It was found that the filtering through the Gooch filters was

greatly facilitated by the addition of 40 to 50 cubic centimeters of 95 percent alcohol to the contents of the flask. The mats were washed five or six times with 50 percent alcohol after being thrown on the filter. The results of this experiment are presented in table 1.

TABLE 1. *Hydrogen-ion Concentration and Growth of Gibberella Saubinetii in Liquid Media in which the Reaction was Adjusted with Sulphuric Acid and Sodium Hydroxide. Temperature, 26° C.*

p_H (Before)	p_H (After)	p_H (Average)	Milligrams Dry Weight
3.85	4.4	4.12	1.6
4.8	3.6	4.2	30.4
5.37	4.5	4.44	54.2
4.8	6.0	5.4	48.0
5.5	5.5	5.5	60.8
5.8	6.2	6.0	60.0
7.5	5.2	6.3	34.8
7.5	5.2	6.35	27.0
7.4	5.6	6.5	36.8
7.5	5.7	6.6	46.8
7 6	5.9	6.75	50.4
.....	7.5	7.5	79.6
8.25	6.9	7.57	74.2

If the average hydrogen-ion concentration expressed as p_H is plotted on the abscissa and the dry weight of the fungus is plotted on the ordinate as in figure 1, it is seen that with decreasing acidity from $p_H = 4.0$ the growth of the fungus increases to a maximum at about 5.5 and then falls sharply to a minimum at 6.3. It then rises again to about 7.5.

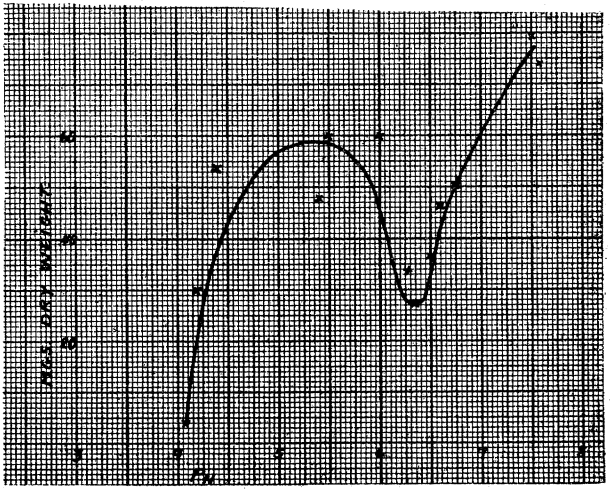

FIG. 1. Relation of hydrogen-ion concentration of liquid culture solution and growth of *Gibberella Saubinetii*—sulphuric-acid-sodium-hydroxide series.

Experiment 2. Growth on Liquid Media—Phosphate Series

In order to check the result reported above, a similar experiment was performed using phosphate solutions to adjust the reaction. Three periods of growth were used in this case instead of one. Four, seven, and fourteen days were selected as suitable lengths of time. At the end of each of these periods a series of cultures was removed and the p_H values and dry weights of the mycelium were determined as before.

The culture solution used contained in one liter:

KNO_3	2.0 g.
$MgSO_4.7H_2O$	0.5 g.
Glucose	10.0 g.

In addition, the solution designated as the "acid solution" contained 9.077 g. KH_2PO_4 per liter, and the "basic solution" contained 11.616 g. of K_2HPO_4 per liter. This made two culture solutions of $M/15$ concentration as regards the acid and basic phosphate respectively. By mixing these two solutions in varying proportion the hydrogen-ion concentrations desired could be obtained. This was roughly accomplished by using the titration curve of Sörensen (14) for phosphate buffer mixtures. For more acid or more alkaline mixtures than could be obtained in this way, $M/15$ concentrations of H_3PO_4 and KOH were prepared in culture solution and added in varying proportions to the "acid" or "basic" solution respectively.

TABLE 2. *Hydrogen-ion Concentration and Growth of Gibberella Saubinetii in Liquid Media in which the Reaction was Adjusted with Primary Potassium Phosphate, Secondary Potassium Phosphate, Phosphoric Acid, and Potassium Hydroxide*

Results after 4 Days		Results after 7 Days		Results after 14 Days	
p_H	Dry Weight (Mg.)	p_H	Dry Weight (Mg.)	p_H	Dry Weight (Mg.)
2.8	0.0	2.77	0.3	2.8	2.2
2.9	0.6	2.9	0.8	3.15	3.6
3.05	0.0	3.1	1.8	3.25	17.2
3.4	1.8	3.47	3.8	3.85	70.6
4.25	6.0	4.6	47.4	5.0	42.2
4.75	4.8	4.6	23.4	5.4	70.4
5.35	5.4	5.07	20.2	5.6	72.2
5.7	7.0	5.45	15.2	5.75	67.8
6.25	9.2	6.25	14.4	5.75	75.0
6.95	3.0	6.85	28.2	7.15	71.1
7.1	7.0	7.1	48.8	7.45	66.1*
7.1	10.6	7.15	31.7[1]	7.55	73.2
7.1	2.8	7.2	13.6	7.7	47.8
7.15	7.8	7.2	3.2	7.75	70.8
7.15	0.8	7.4	2.2	.....	.....
7.2	12.4	7.4	2.2	.....	.....
7.2	3.0	.....	.....	.....	.....

* Average of three determinations.

Inoculation and dry-weight determinations were carried out as before. The cultures were incubated at a temperature of 25.8° C. In table 2 the results at the end of four, seven, and fourteen days are shown. The p_H values are the averages of determinations made at the beginning and end of the period.

The results are expressed graphically in figures 2, 3, and 4. In general the curves are similar in form to the one obtained in the first experiment.

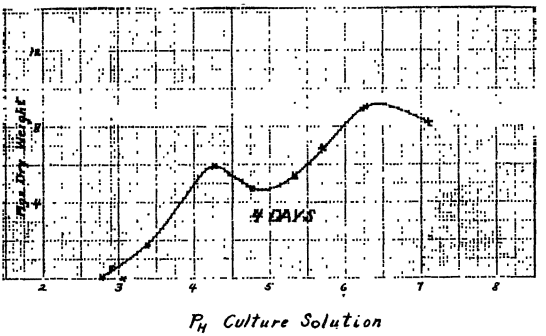

FIG. 2. Hydrogen-ion concentration and growth of *G. Saubinetii* on liquid media—phosphate series after four days.

At the end of four days there is a definite minimum in the curve. After seven days the depression in the curve is more marked, with its lowest point at p_H from 5.5–6.0. In the most acid cultures slight indications of growth were noticed at this time, but the first culture showed no appreciable dry weight. It is interesting to note that in the most acid cultures there were formed a number of colonies, which fact would indicate a stimulation of

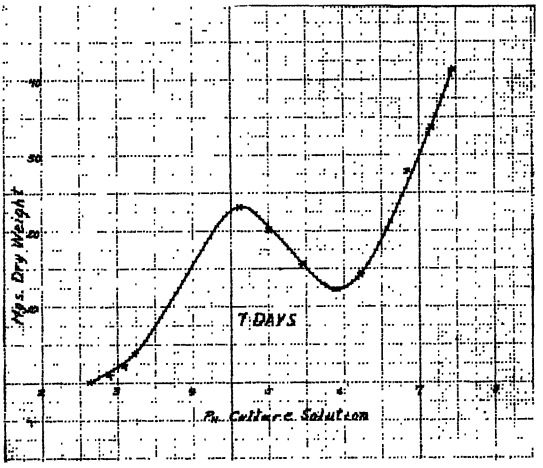

FIG. 3. Hydrogen-ion concentration and growth of *G. Saubinetii* on liquid media—phosphate series after seven days.

conidial production or perhaps segmentation of the mycelium. In the less acid cultures a single colony was usually formed from the original inoculum.

At the end of the fourteen-day period (fig. 4) a depression is still present but it is not so noticeable. At this time the growth curve resembles somewhat the growth curve obtained by Meacham (7) with wood-rotting fungi.

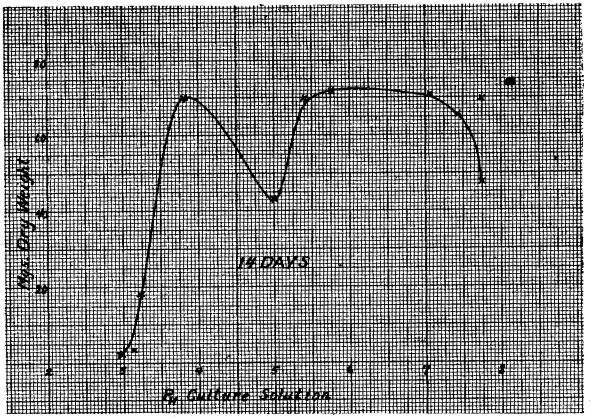

FIG. 4. Hydrogen-ion concentration and growth of *G. Saubinetii* on liquid media—phosphate series after fourteen days.

Experiment 3. Growth on a Solid Medium—Lactic-Acid Series

The growth of the organism was also studied on potato-dextrose-agar plates of varying hydrogen-ion concentration. Lactic acid was used in adjusting the reaction as follows: to 20-cubic-centimeter portions of the melted medium a varying number of drops of lactic acid (50 percent by volume) was added and petri dishes were poured. A separate series of tubes was prepared in the same manner and used for the hydrogen-ion determinations. Each plate was inoculated in five or six places with the mycelium of *Gibberella Saubinetii* on small blocks of agar, and the plates were incubated at 25° C. At the end of 21 and 44 hours respectively, measurements of the diameter of the colonies in millimeters were made. The results at the ends of these two periods are shown in table 3 and represented graphically in figure 5. The diameter given is the average diameter of all the colonies on a plate.

TABLE 3. *Hydrogen-ion Concentration and the Growth of Gibberella Saubinetii on Potato-Dextrose Agar in which the Reaction was Adjusted by Means of Lactic Acid*

Drops of Lactic Acid per 20 Cc. of Medium	P_H	Average Diameter of Colonies after 21 Hours (Mm.)	Average Diameter of Colonies after 44 Hours (Mm.)
0	6.9	18.2	41.2
1	4.5	13.7	32.6
2	4.0	10.4	25.6
3	3.8	9.4	21.0
4	3.6	1.7	10.6

It will be noticed by referring to figure 5 that there is no depression in this curve. This is due to the fact that no acidities intermediate between p_H 4.5 and p_H 6.9 were used. The points at which the minimum occurs were not present in this series, and therefore no minimum was evident. Another

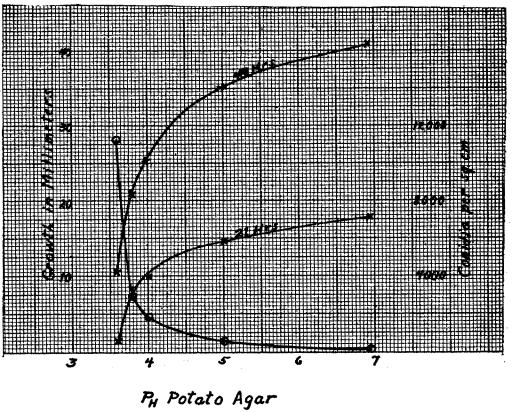

FIG. 5. Hydrogen-ion concentration and growth and conidial production in *G. Saubinetii* on potato-dextrose agar—lactic-acid series.

series with potato agar including acidities between p_H 4.5 and 7.0 demonstrates that a minimum also occurs on potato-dextrose agar. In order to obtain these intermediate values, a solution of lactic acid one eighth the strength of that employed in the former experiment was used. The data for this experiment are given in table 4 and represented graphically in figure 6.

TABLE 4. *Hydrogen-ion Concentration and Growth of Gibberella Saubinettii on Potato-dextrose Agar in which the Reaction was Adjusted by Means of Lactic Acid*

Drops of 50 Percent Lactic Acid per 20 Cc. of Medium	p_H	Average Diameter of Colonies after 19 Hours (Mm.)	Average Diameter of Colonies after 24 Hours (Mm.)	Average Diameter of Colonies after 43 Hours (Mm.)
0	7.3	12.42	17.06	34.26
1/16	7.0	13.86	18.00	34.26
1/8	6.0	14.12	17.32	32.60
2/8	5.7	12.00	15.94	31.30
3/8	5.2	10.50	14.92	30.64
4/8	5.0	12.48	16.28	30.72
6/8	4.65	11.04	14.40	28.48
1	4.4	10.64	13.66	25.74
2	4.0	9.38	11.60	21.00
3	3.8	7.70	9.64	17.30
4	3.6	5.60	7.48	13.78
5	3.5	3.70	5.14	10.50

From the curve in figure 6 it can be noted that a depression in the growth-acidity curve similar to those on liquid media is obtained on potato-dextrose

agar. The deepest minimum appears after 19 hours. After 43 hours there is but a slight depression. The minima appear at a p_H of 5.0–5.5.

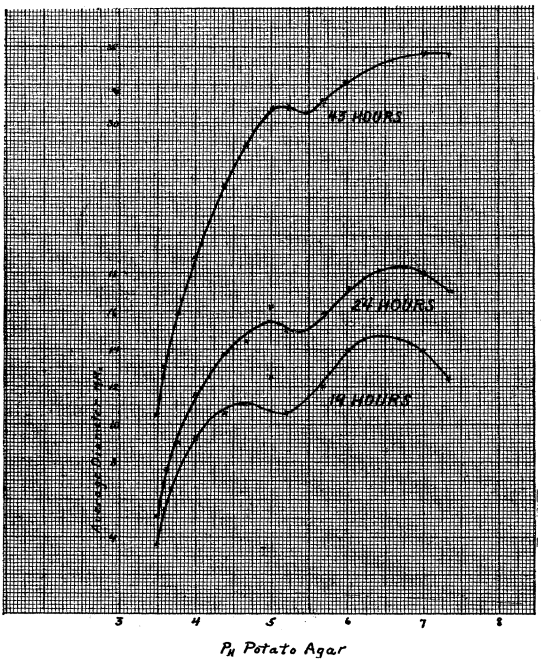

FIG. 6. Hydrogen-ion concentration and growth of *G. Saubinetii* on potato-dextrose agar after nineteen, twenty-four, and forty-three hours—lactic-acid series.

THE RELATION BETWEEN THE HYDROGEN-ION CONCENTRATION OF THE SOIL AND SEEDLING INFECTION OF WHEAT BY GIBBERELLA SAUBINETII

Gillespie and Hurst (3, 4) have investigated the acidity of soils in which potato scab was prevalent and of those relatively free from scab. They found the mean value of the exponent for the Washburn type of soil, on which scab is prevalent, to be p_H 5.93, while that of the Caribou type, which is generally free from scab, was p_H 5.2. This agrees with the results of Gillespie (10), previously discussed, that the growth of the potato-scab organism is inhibited at hydrogen-ion concentrations between p_H 5.2 and p_H 4.8.

More recently Martin (15, 16), in working on the effect of inoculated and uninoculated sulphur on the development of potato scab, determined as well the effect of the sulphur on the hydrogen-ion concentration of the soil. He found that increasing the amounts of sulphur applied to the soil increased the hydrogen-ion concentration of the soil and correlated this increase with the decreasing percentage of scabby tubers. The in-

crease in acidity is due to the oxidation of the sulphur to sulphuric acid. He ascribes the advantage of the inoculated sulphur to its more rapid conversion to the acid.

In the experiments reported in this paper, the effect of hydrogen-ion concentration upon seedling infection of wheat by *Gibberella Saubinetii* is reported. The experiments were carried out under greenhouse conditions in soil in flats. The reaction of the soil was adjusted by means of sulphuric or hydrochloric acid and sodium hydroxide.

Experimental

Soil-Acidity Determinations. These were made according to the method of Gillespie (2). The soils were air-dried and passed through a millimeter sieve. Thirty grams of this soil were placed in a 100-cubic-centimeter centrifuge tube with 30 cubic centimeters of water, the top was closed with the palm of the hand, and the mixture was shaken violently about fifty times. The tube was then centrifuged for about fifteen minutes, and the acidity was determined colorimetrically in the supernatant liquid by means of Gillespie's drop-ratio method. Determinations were checked when possible by using more than one indicator. Gillespie's standards were checked against standard phosphate, phthalate, and acetate buffer mixtures, which were in turn checked by electrometric measurements. The agreement was to 0.1 of a p_H in all cases.

Experiment 4. To a rich loamy soil about one fourth sand was added and the whole was well mixed. Eighteen kilograms were then weighed into each of nineteen flats. The dimensions of the flats were 12 x 18 x 6 inches. A sample of the soil was divided into 250-gram portions, and to each was added a given amount of $N/1$ H_2SO_4 or $N/1$ NaOH made up to a volume of 40 cubic centimeters with water. After mixing the soil and the acid or alkali well and preparing a sample as described above, the hydrogen-ion concentration was determined as described above. From these data a rough preliminary titration curve was constructed. From this curve desired values could be obtained to use in adjusting the reaction of the larger portions of soil. The original soil had a p_H of 5.9. The effects of the acid and alkali on the soil in the flats are shown in table 5.

TABLE 5. *The Effect of Sulphuric Acid and Sodium Hydroxide on the Reaction of the Soil Used in Experiment 4*

Treatment	Cc. $N/1$ H_2SO_4 per 250 G. Soil								Cc. $N/1$ NaOH per 250 G. Soil							
	25	20	15	10	7	3	2	1	0	1	2	5	7	10	15	20	25
p_H	3.4	3.6	3.8	4.4	4.6	5.3	5.5	5.6	5.9	6.4	6.6	6.9	7.6	7.9	8.3	8.6	9.0

The data given in table 5 are also presented in the form of a graph (fig. 7), which is a titration curve of this soil. The acid or alkali added to the flats was thoroughly mixed with the 18 kilograms of soil so that a uniform acidity would be obtained. The soil in seventeen of the flats was adjusted in this manner, two others being retained as checks.

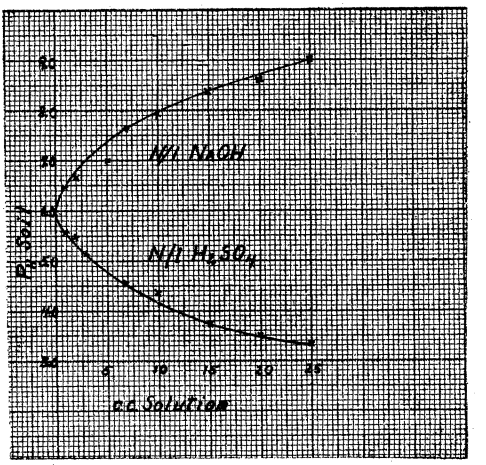

FIG. 7. Titration curve of soil used in experiment 4 with $N/1$ NaOH and $N/1$ H_2SO_4.

The flats were allowed to stand for about a week, when soil samples were taken for acidity determinations. A cork borer was used in taking the sample, and several "cores" of soil were taken from various parts of the flat. They were thoroughly mixed to insure a representative sample. At the end of a week the flats were planted with wheat.

A good sample of Fultz wheat was used. The seed, with the exception of that used in the two control flats, was shaken before planting with a spore suspension of *Gibberella Saubinetii* from a single-spore culture. Approximately 80 seeds were planted in each flat, in two-inch checks, two seeds per hill. The surface of the soil was then sprayed with a spore suspension. A fairly uniform moisture content was maintained by watering the flats with a fine spray from a garden hose. Observations were made of the soil temperature at frequent intervals. This averaged about 20° C. and varied from 20–25° C. A set of soil samples for hydrogen-ion determinations was taken at intervals of one week, four samples in all being taken.

TABLE 6. *The Effect of Soil Reaction on the Germination of Wheat Seedlings*

No. of Seedlings up in 4 Days	3	16	32	33	31	41	24	39	29	38	49	55	34	34	10	0	0
PH	3.65	3.8	4.25	4.55	4.75	5.4	5.6	5.7	5.9	6.4	6.6	7.15	7.45	7.75	8.3	8.65	9.0

Seedlings began to appear in some of the flats at the end of three days, and at the end of four days the number of seedlings in each flat was counted. The number of seedlings with the corresponding p_H values is shown in table 6.

In figure 8 the data of table 6 are shown graphically. This curve is fairly uniform and shows a marked depression at a p_H of about 5.5 with two maxima on either side, one at a p_H of about 4.5 and the other near neutrality (p_H = 7.0).

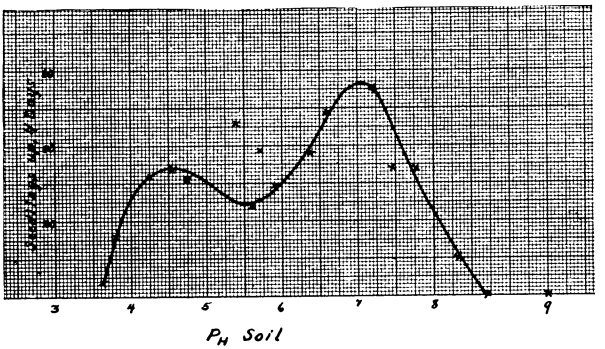

FIG. 8. Relation of number of seedlings up in four days and hydrogen-ion concentration of soil.

It is not certain whether the variations in germination noted in the data in table 6 and in figure 8 are due entirely to the effect of variations in the hydrogen-ion concentration upon infection by *Gibberella Saubinetii*. From experiment 5, however, and also from unpublished data secured by Dr. W. J. Robbins, it is very probable that, apart from infection, variations in the hydrogen-ion concentration cause the curve of the germination of wheat to pass through two maxima with a minimum between.

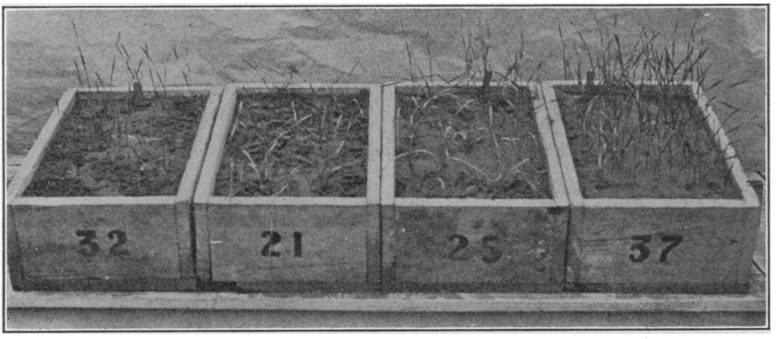

FIG. 9. Soil acidity and seedling infection—the three most acid flats of soil, on left, compared with check flat on right; p_H 3.6, p_H 3.9, p_H 4.4, check.

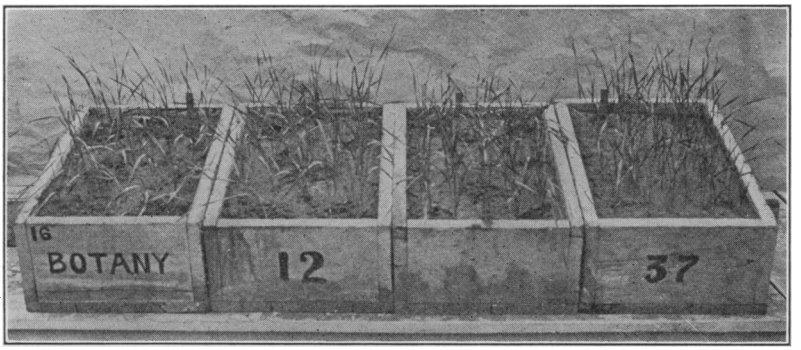

FIG. 10. Soil acidity and seedling infection—p_H 4.65, p_H 4.8, p_H 5.5, check.

Infection in the seedlings was first observed ten days after planting, and frequent counts were made of the total number of seedlings as well as of those known to be infected. Figures 9 to 13 show the appearance of the seedlings as contrasted with one of the uninoculated, untreated control flats. The thinness of stand and the wilting of the plants in the first three flats (the most acid) show a striking contrast to the condition of the control. At a p_H of 5.5 the stand is more nearly perfect, but as we approach the more alkaline soils the stand again becomes poor until at a p_H = 9.0 there are no seedlings up.

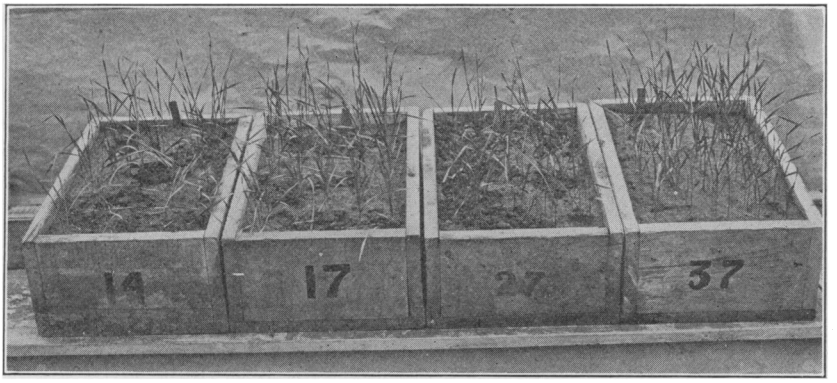

FIG. 11. Soil acidity and infection—p_H 5.7, p_H 5.8, p_H 5.9, check.

At the end of three weeks all seedlings were removed and the number of infections was recorded. Many seedlings which appeared healthy were found to be infected below the surface of the soil. In the most acid and most alkaline soils many seedlings which had germinated were rotted before reaching the surface of the soil. In most cases the greater amount of injury was due to stem rot, although in the very alkaline soils more root rot was observed.

FIG. 12. Soil acidity and infection—pH 7.2, pH 7.5, pH 7.8, check.

Re-isolations were made from several typical lesions from each flat, and pure cultures of *Gibberella Saubinetii* were invariably obtained. The two control flats containing the original uninoculated soil showed a perfect stand and no diseased seedlings. In table 7 a summary of the data is presented. These data are also shown graphically in figure 14. For an experiment of this type the curve is very uniform. A maximum appears at $p_H = 4.0$, followed by a clear-cut minimum at $p_H = 5.5$. As the more alkaline soils are reached the curve rises, and in the most alkaline infection is as high as 100 percent.

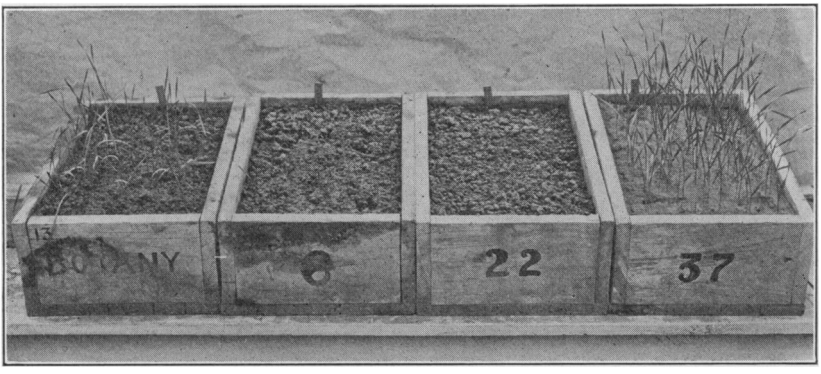

FIG. 13. Soil acidity and infection—pH 8.2, pH 8.6, pH 9.0, check.

Experiment 5. In the above described experiment a control or uninoculated soil was not used for each soil acidity, and the depression in the curve might be caused by the sulphate ion. A second experiment was therefore performed in which hydrochloric acid was used in place of sulphuric acid in adjusting the soil reaction, and a flat of uninoculated soil at each acidity was used as a check.

TABLE 7. *Hydrogen-ion Concentration of the Soil and Seedling Infection. Sulphuric-acid Series*

No. of Flat	pH (Average)	Total Plants	Number Infected	Percent Infected
32	3.63	76	43	56.6
21	3.90	77	63	81.8
25	4.36	83	63	75.9
16	4.65	70	42	60.0
12	4.80	76	32	42.1
35	5.53	82	19	23.2
14	5.66	70	39	55.7
17	5.80	73	29	39.7
27	5.90	75	41	54.7
15	6.47	80	43	53.8
29	6.67	84	44	52.4
11	7.22	79	42	53.2
31	7.48	82	54	65.9
10	7.78	79	39	49.4
13	8.22	79	72	91.2
6	8.63	79	79	100.0
22	9.06	77	77	100.0
37	Control	80	0	0
7	Control	81	0	0

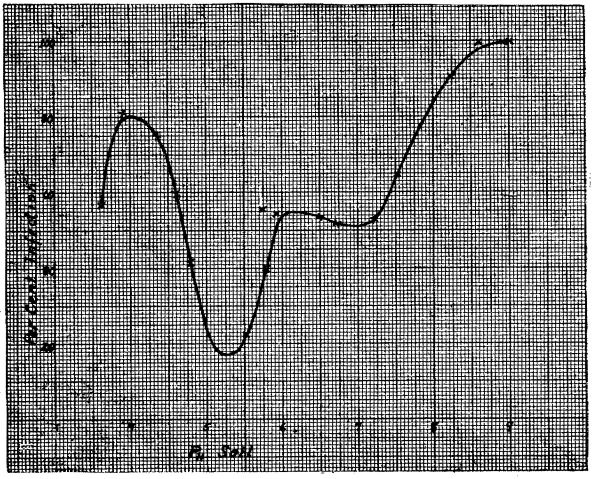

FIG. 14. Soil acidity and infection—hydrogen-ion concentration in its relation to the percentage of seedling infection—sulphuric-acid series.

Another soil sample was used. Its reaction was adjusted in essentially the same manner as before. Using *2N* HCl and *2N* NaOH, a titration curve of this soil was constructed and the quantities of acid or alkali required to secure the desired acidities were calculated for 36 kilograms of soil. After thorough mixing, the soil was divided between two flats, one to be inoculated and the other for a control. The titration data which are given in table 8 are the results obtained in the flats after mixing the 36 kilograms of soil in each case with the required amount of solution. The

results are expressed, however, on the basis of 250 grams of soil. A curve constructed from these data is shown in figure 15.

TABLE 8. *The Effect of Hydrochloric Acid and Sodium Hydroxide on the Reaction of the Soil Used in Experiment 5*

Treatment	Cc. 2N HCl per 250 G. of Soil											Cc. 2N NaOH				
	13	10	7	5	4	3.5	3	2.5	2	1.5	1	0	2	5	10	13
pH.	3.5	3.8	4.47	4.25	5.12	5.3	5.43	5.47	5.47	5.6	5.74	6.4	6.98	7.18	7.83	7.83

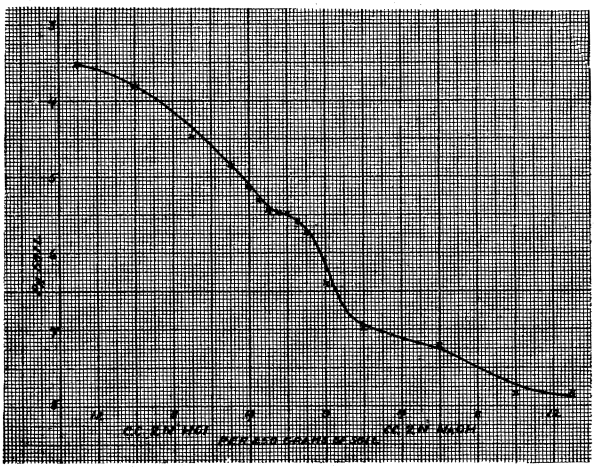

FIG. 15. Titration curve of soil used in experiment 5 with 2N HCl and 2N NaOH.

Inoculation and planting were carried out as in experiment 4. The seeds planted in the sixteen uninoculated flats were soaked in sterile water for the same length of time that the others were immersed in the spore suspension.

The appearance of the seedlings at the surface of the soil was first noted three days after planting. A larger number of seedlings was found in the inoculated series. In the latter series seventeen seedlings were counted, as compared with seven in the inoculated series. The total number of seedlings up in the uninoculated flats continued to be larger than in the inoculated set. The relation between the two series and the relation of each to the acidity are brought out in table 9 and also in the graphs in figures 16 and 17. At this time it is not certain whether or not this difference is due to infection by *Gibberella Saubinetii*. The p_H values given are the averages of two series of determinations made one day before and four days after this period respectively.

TABLE 9. *Relation of Soil Acidity to the Number of Seedlings Up in Four Days in the Inoculated and Uninoculated Series*

Seedlings up 4 Days Controls	0	0	0	1	4	12	1	2	4	16	29	57	53	38	5	9
Seedlings up 4 Days —Inoculated Series	0	0	0	0	0	0	2	4	0	15	21	26	27	30	1	0
PH	3.42	3.8	4.35	4.56	4.89	5.25	5.37	5.4	5.47	5.57	5.71	6.3	6.91	7.25	7.8	7.82

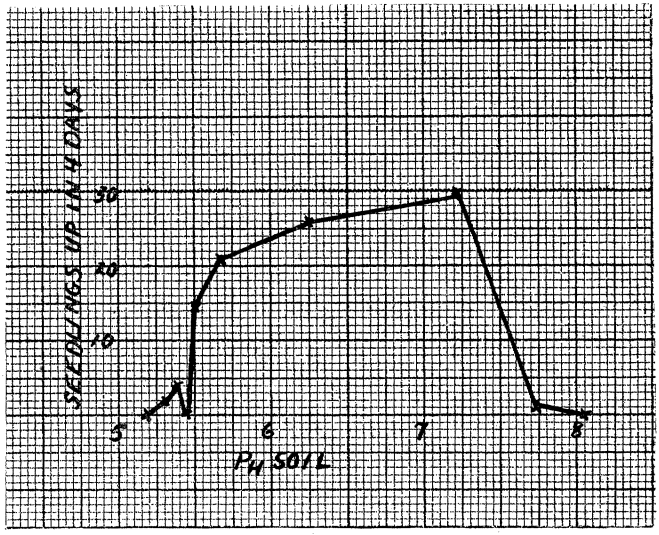

FIG. 16. Hydrogen-ion concentration of soil and number of seedlings up in four days in inoculated flats. Hydrochloric-acid series.

Although there is only slight evidence of a minimum in the inoculated series, due perhaps to unfavorable greenhouse conditions at the time this experiment was run, the uninoculated series shows a distinct minimum at about p_H 5.5. This indicates not only that the phenomenon of this minimum is related to infection, but that there is an independent action of the soil acidity on the germination of wheat.

The first signs of infection at the surface of the soil were noted nine days after planting. The final data were taken at the end of three weeks. The plants were all removed as in experiment 4, and the percentage of infection was determined. These data are presented in table 10 and are shown graphically in figure 18. The p_H values in this case are the averages of three series of determinations. The temperature during this experiment varied from about 12° C. to 30° C., with an average of about 19.5° C.

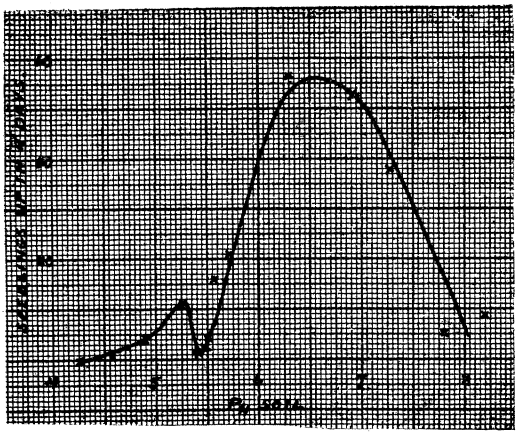

FIG. 17. Hydrogen-ion concentration of soil and number of seedlings up in four days in uninoculated flats. Hydrochloric-acid series.

TABLE 10. *Hydrogen-ion Concentration of the Soil and Seedling Infection. Hydrochloric-acid Series*

No. of Flat	p_H (Average)	Total Plants	Number Infected	Percent Infected
33	3.5	67	48	71.6
42	3.8	50	21	42.0
40	4.47	65	50	76.9
49	4.85	58	44	75.9
56	5.12	62	37	59.7
41	5.3	61	39	64.0
38	5.43	66	31	47.0
55	5.47	69	28	40.5
28	5.47	63	22	34.9
45	5.6	70	25	35.7
18	5.74	78	32	41.0
24	6.4	79	38	48.1
1	6.98	80	39	48.7
3	7.18	75	40	53.3
46	7.83	58	45	77.6
43	7.83	69	52	75.3

In the uninoculated flats an occasional infected seedling was found, due perhaps to natural infection from the seed. All others were uninfected at the time of digging. Examining the data given in table 10 as shown in figure 18, it can be seen that when hydrochloric acid is used in place of sulphuric acid in the adjustment of the soil to various acidities, the same phenomenon occurs as was noted in experiment 4. A minimum in the infection is present at a p_H of 5.5. This is strong evidence that the minimum in the infection curve is due to the hydrogen-ion concentration alone. This is strengthened of course by the results obtained in the growth of *Gibberella Saubinetii*, when an adjustment of the reaction with sulphuric acid, acid

phosphate, and lactic acid gave similar low points in the growth-acidity curve.

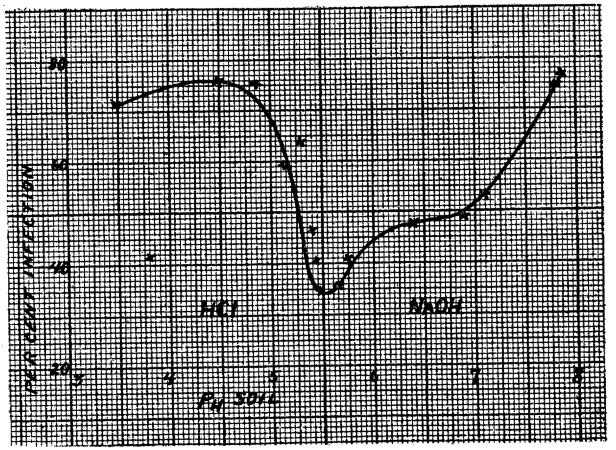

FIG. 18. Soil acidity and infection—hydrogen-ion concentration in its relation to the percentage of seedling infection. Hydrochloric-acid series.

SUMMARY

The experiments reported in this paper were undertaken to determine the relation of hydrogen-ion concentration to wheat scab. It was desired to find if possible a limiting acidity for seedling infection of wheat by the causal organism of this disease, *Gibberella Saubinetii*.

A study of the pathogene *Gibberella Saubinetii* shows that, although a wide range of acidity is tolerated, there is a minimum in the growth curve. This minimum in the curve was found to be present in three different series of cultures. In the first series the reaction of a liquid medium was adjusted by means of sulphuric acid and sodium hydroxide; in the second, primary and secondary potassium phosphate, phosphoric acid, and potassium hydroxide were used in a liquid medium, and in the third, the acidity of potato-dextrose agar was varied by means of lactic acid. The minimum point in the curve varied from about p_H 5.5 to p_H 6.0. This is similar to the results of Webb (13) in his work on spore germination and hydrogen-ion concentration. The use of various substances to change the reaction shows that the effect on the growth is due to the hydrogen-ion concentration and not to other molecules or ions.

An interesting correlation appears in the relation of soil acidity to seedling infection. Here also a minimum was obtained in two cases at p_H 5.5. In one instance the reaction was adjusted by means of sulphuric acid and sodium hydroxide, and in the other hydrochloric acid and sodium hydroxide were used. It seems, therefore, that there is a relation between the effect of acidity on the growth of the pathogene and its effect on in-

fection. Furthermore, it appears from results obtained on the effect of acidity on the rate of germination of wheat in the control flats, which also shows a minimum, that there is as well an effect of the hydrogen ion on the host, which causes a depression in the infection curve. How this comes about is not certain, but it seems plausible that both these phenomena affect the severity of the infection. Nor is it at all clear at present what the cause of the depression in these curves is. In the opinion of the writer this will be solved only by further study of hydrogen-ion concentration in its relation to other factors.

Because of the great importance not only of wheat scab, but of fusarial diseases as a whole, it is hoped that the results obtained in this study may have a practical bearing in the control of such soil-borne pathogens. Attention is directed to the fact that the minimum in the infection curve occurs at p_H 5.5, a not unusual soil reaction to which the soil could easily be adjusted.

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